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(54) Title: METHOD FOR ASSESSING THE RELATIVE PURITY OF VIRAL GENE TRANSFER VECTOR STOCKS
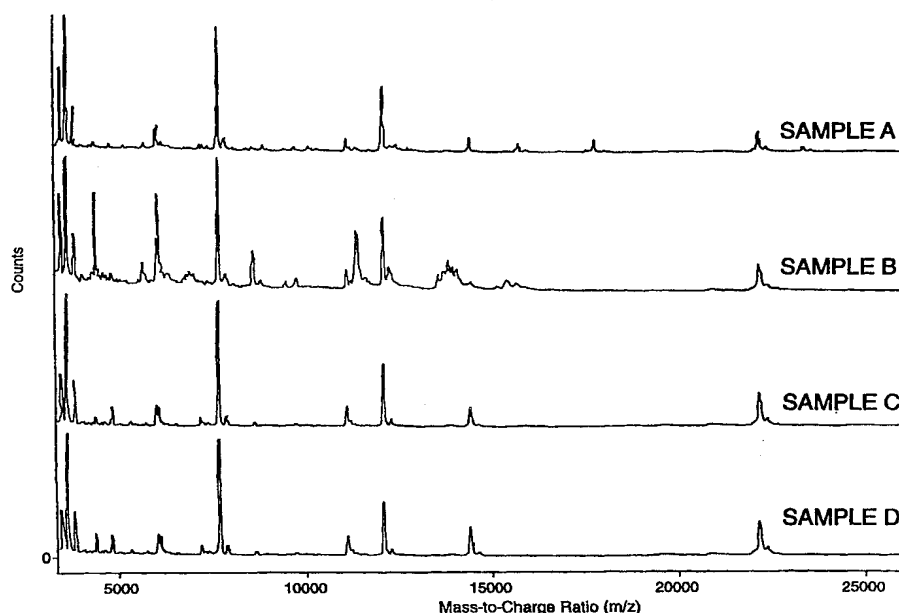
(57) Abstract

The present method involves evaluating the purity of a viral gene transfer vector stock by providing an aliquot of the viral gene transfer vector stock and producing charged fragments from the aliquot. The fragments are detected by a detector that produces a sample signal corresponding to the mass–to–charge ratio of the detected fragments. This signal then is compared to a standard signal, whereby the purity of the viral gene transfer vector stock is evaluated.

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METHOD FOR ASSESSING THE RELATIVE PURITY OF VIRAL GENE
TRANSFER VECTOR STOCKS

TECHNICAL FIELD OF THE INVENTION

5   The present invention relates to methods of
assessing the relative purity of stocks of viral gene
transfer vectors.

BACKGROUND OF THE INVENTION

10   Viral gene transfer vectors are widely used
throughout molecular biology, especially in gene therapy.
A major problem posed by the use of viral gene transfer
vectors for gene therapy is the lack of consistent and
effective methods to assess the purity of a stock of
15   viral gene transfer vector. The lack of efficient means
for determining purity of viral gene transfer vectors has
resulted in the inability to measure the effectiveness or
consistency of viral gene transfer vector production
techniques. These problems are magnified when
20   considering the safety concerns surrounding use of viral
gene transfer vectors in human gene therapy.

       Traditionally, attempts to measure the purity of an
unmodified virus particle in a medium have relied on
electrophoresis techniques, such as sodium dodecyl
25   sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), or
ultraviolet (UV) spectroscopy measurements. Capillary
gel electrophoresis also has been used to analyze viral
components in comparison to known standards, as
illustrated in U.S. Patent 5,723,031 (Durr et al.) In
30   addition, ultraviolet spectroscopy techniques and
ultraviolet resonance spectra have been used to provide
structural analyses of several viruses.

       Other techniques for assessing viral purity have
centered on the use of chromatography techniques such as
35   ion exchange chromatography, gel-filtration
chromatography, and reverse phase high performance liquid
chromatography (HPLC). For example, researchers have

utilized HPLC to purify structural proteins from Herpes
Simplex Virus type-1. Similar techniques for purifying
viral components have been developed using cation
exchange chromatography as described in U.S. Patent

5   5,486,470 (Darke et al.), ion exchange chromatography as
described in U.S. Patent 5,696,238 (Haigwood and
Scandella), and electrical chromatography as described in
U.S. Patent 5,707,850 (Cole and Gaithersburg).

Mass spectrometry, with the advantage of fast,
10 accurate and detailed analysis as compared to several of
the aforementioned techniques, has been used to study
individual organic compounds. For example, Vastola et
al., *Organic Mass Spectrometry, 3,* 101-104 (1969),
disclosed the use of laser pyrolysis mass spectrometry to
15 study the spectra generated by organic salts. Since that
time, mass spectrometry has been widely used to evaluate
features of larger organic molecules such as proteins.
Recently these techniques have been used to study or
validate the purity of individual viral proteins or
20 groups of viral proteins such as the components and
modifications of viral capsid proteins. For example,
Fetzer et al., *Protein Expression and Purification, 5,*
432-441 (1994), teach the use of mass spectrometry in
combination with SDS-PAGE to validate the purity of a
25 herpes simplex virus thymidine kinase protein that was
subjected to proteolytic cleavage and chromatography
purification.

Other researchers have studied groups of viral
proteins, for example, Davidson and Davidson, *Virology,*
30 *206,* 1035-1043 (1995), disclosed the identification of
proteins of channel catfish virus by use of fractionation
techniques in combination with laser desorption
ionization mass spectrometry. Similarly, Pepinksy et
al., *J. Virology, 70,* 3313-18 (1996), used electrospray
35 mass spectrometry to obtain molecular masses for *Gag*
proteins in the Rous sarcoma virus, to study protein

intermediates, and to determine that exopeptidase
trimming occurred in some of the *Gag* proteins.

More recently, Bothner et al., *J. Biol. Chem.*, *273*,
673-76 (1997), have disclosed the use of matrix assisted
5   laser desorption/ionization mass spectrometry (MALDI or
MALDI-MS), in combination with limited proteolysis, upon
flock house virus particles to study viral capsid
dynamics in that species. Also recently, mass
spectrometry has been used as a tool for diagnosing
10   specific DNA molecules in viruses, as described in U.S.
Patent 5,605,798 (Koster).

Not until very recently has mass spectrometry been
applied to entire virus particles. Tas et al.,
Biomedical and Environmental Mass Spectrometry, *18*, 757-
15   760 (1989), met with mixed results in attempting to
characterize Vero cell cultures infected with herpes
simplex virus and poliomyelitis virus by studying low-
weight molecules (i.e., salt molecules, methyl groups,
and sugars) that were detected by use of pyrolysis/direct
20   chemical ionization (Py/DCI) mass spectrometry. While
Tas et al. suggested that they could possibly identify
cell lines infected with a particular virus, they failed
to teach a method of obtaining relatively pure viral
stocks, especially of a viral gene transfer vector
25   capable of use in molecular biological applications,
particularly gene therapy.

Despeyroux et al., *Rapid Communications in Mass
Spectrom.*, *10*, 937-41 (1996), were able to generate a
spectrum for purified cricket paralysis virus (CrPV) by
30   using electrospray ionization mass spectrometry and
suggested that comparisons could be made to such spectra.
However, Despeyroux et al.'s results are limited because
success of their technique was attributed to the small
size and simplicity of structural polypeptides of the
35   cricket paralysis virus. Furthermore, similar to Tas et
al., Despeyroux et al. failed to teach the combination of
such techniques with a useful viral gene transfer vector.

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Siuzdak et al., *Chemistry & Biology*, *3*, 45-48
(1996), similarly used electrospray ionization (ESI) mass
spectrometry, under special settings, to detect viruses.
Using standard ESI techniques, Siuzdak et al. were not
5   able to derive signals for large virus particles.
Siuzdak et al. were capable of generating signals and
performing mass selection on the charged particles from
tobacco mosaic virus (TMV) and rice yellow motile virus
(RYMV), by using the radio frequency (RF) mode of an ESI
10   mass spectrometer in a manner that retained the
ultrastructure of the virus particles. Thus, Siuzdak et
al. taught that non-destructive ESI could actually purify
virus particles. However, Siuzdak et al. only reported
on small viruses (i.e., molecular weights of less than
15   50,000,000 Daltons), and did not assess the relative
purity of a virus that had not been modified by
subjection to mass spectrometry.

Siuzdak, *J. Mass Spectrom.*, *33*, 203-211 (1998), also
disclosed that TOF mass spectrometry might be used to
20   measure large ions such as a whole Tobacco Mosaic Virus.
However, such work, if carried out, was not published,
and nevertheless would not have provided a method of
easily and efficiently assessing relative viral purity,
especially in regard to large virus particles, such as
25   with a stock of a useful viral gene transfer vector.

Moreover, many specific types of viral gene transfer
vectors that have proven to be powerful tools in
molecular biology often have molecular weights well in
excess of those of TMV and RYMV. For example, adenoviral
30   vectors, which are useful delivery vehicles, such as in
gene therapy, have molecular weights often exceeding
150,000,000 Daltons, three times the weight of the
viruses studied by Siuzdak et al.

Furthermore, the techniques taught by Tas et al.,
35   Despeyroux et al., and Siuzdak (i.e., ESI and Py/DCI mass
spectrometry) are difficult to perform, and require
complicated analysis to evaluate their results.

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Therefore, such techniques may not be a viable
alternative for widespread use in the production of viral
gene transfer vector stocks.

5   In view of the state of the art, there remains a
need for methods to efficiently evaluate the purity of
stocks of viral gene transfer vector. More particularly,
there is a need to evaluate the relative purity of stocks
of viral gene transfer vectors, suitable for carrying
large amounts of genetic information for use in practical
10   biological applications.

                    BRIEF SUMMARY OF THE INVENTION
       The present invention provides a method of
evaluating the purity of a viral gene transfer vector
15   stock. The present method of evaluating the purity of a
viral gene transfer vector stock comprises providing an
aliquot of a viral gene transfer vector stock and
producing charged fragments from the aliquot. The
fragments are detected by a detector that produces a
20   sample signal corresponding to the mass-to-charge ratio
of the detected fragments. This signal then is compared
to a standard signal, whereby the purity of the viral
gene transfer vector stock is evaluated.

25   BRIEF DESCRIPTION OF THE DRAWINGS
       Figure 1 is a graph of mass spectrometer traces of
various viral gene transfer vector stocks plotted as
mass-to-charge ratio (m/z) (x-axis) versus relative
counts (y-axis).
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               DETAILED DESCRIPTION OF THE INVENTION
       The present method involves evaluating the purity of
a viral gene transfer vector stock by providing an
aliquot of a viral gene transfer vector stock and
35   producing charged fragments from the aliquot. The
fragments are then detected by a detector, which produces
a sample signal corresponding to the mass-to-charge ratio

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of the detected fragments. A standard signal is
provided, and the purity of the viral gene transfer
vector stock is evaluated by comparing the sample signal
to the standard signal.

5   The present invention is particularly useful in the
context of purifying (i.e., substantially enriching a
solution comprising) a viral gene transfer vector, for
example, a recombinant adenoviral gene transfer vector.
       The viral gene transfer vector can be any viral
10   vector that contains at least one exogenous gene. A
vector is any polynucleotide competent for introducing
one or more exogenous genes into a cellular environment.
One skilled in the art will recognize that the viral gene
transfer vector (i.e., the viral vector as well as the
15   exogenous gene) can be composed of single-stranded
ribonucleic acid (RNA), double-stranded RNA, single-
stranded deoxyribonucleic acid (DNA), or double-stranded
DNA.
       The viral vector can be any vector capable of
20   containing recombinant nucleic acids that are (at least
in part) of viral origin. A viral vector as indicated
above can be any virus which contains DNA, for example,
an adenoviral vector, or one that contains RNA, for
example, a retrovirus. Examples of such vectors include,
25   but are not limited to, adenoviral vectors (Bain et al.,
Gene Therapy, 1, S68 (1994)), adeno-associated viral
vectors (Berns et al., *Annals of the New York Academy of
Sciences,* 772, 95-104 (1995)), packaged amplicons
(Fereroff et al., *Proc. Nat. Acad. Sci. USA,* 89, 1636-40
30   (1992)), and herpes simplex virus-based viral vectors
(Fink et al., *Ann. Rev. Neurosci.,* 19, 265-87 (1996)).
       Preferably, a viral vector is a vector derived from
viral origin that contains nucleic acid sequences which
encode for (a) the production of a capsid, a partial
35   capsid, modified capsid, capsid protein, or modified
capsid protein, any of which may or may not have all of
the protein sub-units or sub-unit arrangement of the wild-

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type viral capsid that corresponds to the origin of the
viral vector or even identical sub-units to those
expressed in the wild-type virus, (b) the functional
portion of at least one terminal repeat, for example, the
5   inverted terminal repeats found in adenoviruses or the
long terminal repeats found in retroviruses, preferably
two functional terminal repeats, including, but not
limited to, long terminal repeats (LTRs) or inverted
terminal repeats (ITRs) exhibited in viruses which assist
10   in DNA replication, and (c) at least a partial viral
packaging sequence, more preferably a sequence that
enables the packaging of reproduced viral vector nucleic
acids that are about 73% to 110% in size of the
corresponding wild type viral genome.
15   More preferably, the sequences encoding for a capsid,
partial capsid, modified capsid, capsid protein(s), or
modified capsid protein(s) encode a protein arrangement
that substantially shields the viral vector DNA from
damage or degradation by enzymatic or other means.
20   The viral gene transfer vector can have any suitable
molecular weight. Viral gene transfer vectors of
substantial molecular weights (e.g., the combined mass of
proteins, lipids, polysaccharides, and nucleic acids,
including the exogenous gene, that comprise the viral gene
25   transfer vector) can be evaluated within the context of
the present method. For example, the viral gene transfer
vector can possess a molecular weight of at least
1,000,000 Daltons, 3,000,000 Daltons, 20,000,000 Daltons,
50,000,000 Daltons, 100,000,000 Daltons, 150,000,000
30   Daltons, 500,000,000 Daltons, 1,000,000,000 Daltons, or
even 1,500,000,000 Daltons.
           Similarly, the viral gene transfer vector can have
any suitable genome size comprised of nucleic acid
sequences or base pair sequences native to the viral
35   vector and those that comprise the exogenous gene or
genes. Viral gene transfer vectors of large genomes can
be evaluated within the context of the present method.

For example, the viral gene transfer vector can have a
total genome of at least 2,000 nucleotides or nucleic acid
base pairs, 4,000 nucleotides or nucleic acid base pairs,
7,000 nucleotides or nucleic acid base pairs, 20,000
5   nucleotides or nucleic acid base pairs, 30,000 nucleotides
or nucleic acid base pairs, or even 100,000 nucleotides or
nucleic acid base pairs.

    The exogenous gene in the context of the present
invention includes any exogenous nucleic acid that
10   encodes a desired polypeptide or ribonucleic acid, is
operatively linked to a suitable promoter, and can be
expressed to produce the desired polypeptide or
ribonucleic acid. For example, the exogenous gene can
comprise a nucleotide sequence that can be expressed to
15   produce a polypeptide, a ribozyme, or antisense RNA
molecule. The exogenous nucleic acid is not native to
the viral vector, although the promoter operatively
linked thereto may or may not be native to the viral
vector. Thus, the viral gene transfer vector is not a
20   wild-type virus. The polypeptides or ribonucleic acid
encoded by the exogenous nucleic acid can be any suitable
polypeptide or ribonucleic acid, such as a reporter
polypeptide or ribonucleic acid (i.e., the exogenous gene
can be a reporter gene), and preferably a therapeutic
25   polypeptide or ribonucleic acid (i.e., the exogenous gene
can be a therapeutic gene).

    The present invention involves the evaluation of a
stock of the viral gene transfer vector. The viral gene
transfer vector stock can be any composition comprising a
30   viral gene transfer vector and a suitable carrier
therefor. The choice of carrier will be determined in
part by the particular end use of the viral gene transfer
vector stock. The carrier preferably is a
pharmaceutically acceptable carrier. A pharmaceutically
35   acceptable carrier typically will be a substance useful
in the administration of the viral gene transfer vector
to an animal, such as a human, for therapeutic treatment.

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Pharmaceutically acceptable carriers are known to those
skilled in the art and are readily available.

A viral gene transfer vector stock can be produced
by transfecting a viral gene transfer vector into a cell
5   (i.e., a production cell) that is permissive for the
replication of the viral gene transfer vector to produce
a transfected cell. The permissive cell is ordinarily
maintained in a medium that promotes cell growth,
examples of which are widely known in the art. The
10   transfected cell is maintained in the medium so as to
cause the viral gene transfer vector to replicate. The
viral gene transfer vector will ordinarily be separated
from the cell and from the medium to produce a stock of a
viral gene transfer vector.
15   Preferably, the viral gene transfer vector stock is
obtained by infecting eukaryotic cells with a viral gene
transfer vector of interest, maintaining the cells for a
period of time sufficient to amplify the number of viral
gene transfer vector particles, gathering the infected
20   cells together, and lysing (i.e., breaking open) the
cells in a buffered solution. The viral gene transfer
vector stock can be subsequently treated or modified in
any suitable manner. For example, purification or
separation techniques can be performed on the viral gene
25   transfer vector stock to remove the viral gene transfer
vector from the cells and the medium, thereby yielding a
more concentrated stock of the viral gene transfer
vector. Such purification and separation techniques are
well known in the art. Such techniques can include, but
30   are not limited to, chromatography, electrophoresis,
enzymatic or chemical treatment, exposure to heat or
radiation, centrifugation, and separation by a
differential gradient, for example, a cesium chloride
gradient.
35   The viral gene transfer vector stock can have any
suitable level of purification or concentration. For
example, the present method can be practiced on

unmodified stocks of viral gene transfer vectors which
can include undesired substances or molecules, as well as
stocks that are considered substantially pure. More
specifically, such undesired substances or molecules can
5   include, but are not limited to, the cell, other
components of the stock, for example, an undesired
buffering agent, and inorganic and organic molecules or
polymers, such as biomolecules from the viral gene
transfer vector or other living organisms or virus
10   particles, such as nucleic acids, lipids,
polysaccharides, and proteins.

     The production cell can be any cell permissive of
the reproduction of the viral gene transfer vector. The
production cell preferably is a eukaryotic cell, more
15   preferably a mammalian cell, and most preferably a human
cell. The production cell can be a primary or secondary
cell or can originate from a cell line, for example, an
immortalized cell line, or a cell line that expresses an
immortalized phenotype or characteristics. Such
20   characteristics can be used as a marker, for example, by
selecting cells that exhibit such characteristics.
Alternatively, or in combination, the production cell can
be a cell that expresses a marker gene that allows for
either positive or negative selection based upon the
25   expression of the marker gene.

     The production cell can have any morphological
characteristics that allow reproduction of the viral gene
transfer vector in that particular cell. For example,
the production cell can be epithelial or epithelial-like
30   in morphology, such as cells of, or derived from, human
kidney, lung, or neural cells. The production cell also
can be an embryonic cell, preferably a human embryonic
cell, for example, a human embryonic kidney cell, human
embryonic lung cell, or human embryonic retinoblast.

35   The production cell can be a cell that has been
modified to express nucleic acids that partially *trans-*
complement the viral gene transfer vector, especially

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when the viral gene transfer vector otherwise is
replication incompetent. For example, the production
cell can be a cell that *trans*-complements a replication
incompetent viral gene transfer vector so as to prevent
5   undesired recombination between the genome of the
production cell and the viral gene transfer vector, e.g.,
to prevent a homologous recombination event between the
genome of the production cell and the viral gene transfer
vector, while allowing the reproduction of the viral
10   vector in the production cell.

        An aliquot can be taken from the viral gene transfer
vector stock by any suitable technique. Charged
fragments (e.g., ionized fragments) are produced from the
aliquot by any suitable technique. If desired, the
15   aliquot can be appropriately treated prior to production
of the charged fragments therefrom. For example, the
aliquot can be treated to reduce the salt level therein
(e.g., from a physiological level of 150 mM salt to 50 mM
salt or less) to reduce background noise in subsequently
20   detecting the fragments, even if the viral gene transfer
vector stock itself will not be so treated, so long as
the subsequent comparison of the sample and standard
signals is not adversely affected. The present method
can be performed using techniques that allow
25   fragmentation without the use of chemical ionization
methods. For example, the present technique does not
require the use of pyrolysis direct/chemical ionization
(PY/DCI) mass spectrometry, or other forms of chemical
ionization mass spectrometry, to produce suitable charged
30   fragments.

        While the charged fragments can be produced by any
suitable technique, the charged fragments typically will
be produced by contacting the aliquot with radiation,
electrons, or protons, preferably protons. Any suitable
35   form of radiation, electrons, or protons can be used in
the present method to produce charged fragments from the
aliquot. Radiation as used in the context of the present

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invention refers to the emission of energy in the form of electromagnetic waves, acoustic waves, or particles. Examples of radiation suitable in the context of the present invention include, but are not limited to, radio waves, microwaves, visible light, ultraviolet (UV) light, far UV and infrared rays, x-rays, gamma-rays, infrasonic waves, sonic waves, ultrasonic waves, and α- and β-rays of radioactivity. The electrons can be in any suitable form. For example, the electrons can be in the form of electron beams or individual electrons. Similarly, the protons can be in any suitable form.

The radiation, electrons, and protons can be from any suitable source. For example, radiation can be emitted from a natural source (e.g., radioactive cobalt), an x-ray device, or a laser.

The radiation, electrons, and protons can have any suitable characteristics. In particular, the radiation can have any suitable wavelength. For example, the method can incorporate a radiation pulse that has a wavelength in the infrared spectrum or the ultraviolet spectrum. Similarly, any pulse width suitable to produce fragments can be utilized in the present method. For example, the pulse generated by a laser can have a width of up to about 100, 200, or even 5,000 nanoseconds. Moreover, variable pulse widths and multiple repeated pulses can be used to produce fragments.

The produced fragments can be in any state or quantity suitable for fragment detection. For example, the aliquot can be vaporized at the time the charged fragments are produced from the aliquot, such that the fragments enter into a gaseous or vapor state, have greater mobility, and are subject to easier detection. The fragments can have any suitable velocity at the time of production that allows for detection by the detector. For example, fragments can be accelerated, after they are produced, to a desired velocity before the fragments are detected. The fragments can pass through a field-free

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region, wherein the velocity of the fragments within the
field-free region is proportional to the mass-to-charge
ratio of the fragments. One example of such a field-free
region is an electric field, particularly an electric
5   field wherein lighter fragments have a higher velocity
than heavier fragments. While acceleration of fragments
is not required, if the fragments are accelerated, any
level of acceleration sufficient for detection of the
fragments is suitable. For example, the fragments can be
10   accelerated to a fixed kinetic energy by contacting the
fragments with an electric potential.

          A viral vector typically will comprise proteins that
are associated (or bound) to each other, as well as
nucleic acids, which together form the ultrastructure of
15   the viral vector. As a result of contact with the
protons, the proteins can become disassociated from one
another (but otherwise remain substantially intact)
and/or the proteins can be further fragmented to yield
portions of the proteins. A significant amount of
20   information can be obtained about the viral gene transfer
vector stock by fragmenting the individual proteins into
portions of the proteins; however, the analysis of such
information can be complex and time-consuming. It has
been found that the mere disassociation of the viral
25   vector proteins, e.g., merely causing the viral gene
transfer vector to lose its ultrastructure through the
disassociation of, for example, viral capsid or modified
capsid proteins, such that the produced fragments of the
viral gene transfer vector comprise, preferably consist
30   essentially of, and more preferably consist of,
substantially or entirely intact viral vector proteins
typically is sufficient for purposes of present invention
and provides information that can be easily and quickly
analyzed.

35   The detection of the produced fragments can be
accomplished by any suitable technique. The detection
step can include, for example, measuring the time-of-

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flight for the detected fragments, wherein the time-of-
flight is the approximate time required for a fragment to
travel a distance across a field-free region. A signal
corresponding to the time-of-flight then can be
5   generated.

        While the fragmentation and detection steps of the
present method can be performed in any suitable manner,
they are preferably performed by the use of an analytical
device, most preferably by the use of an ion source and a
10   mass analyzer, such as are present in mass spectrometers.
Suitable ion sources include electron impact, fast ion or
atom bombardment, field desorption, laser desorption,
plasma desorption (PD), thermospray (TSP), electrospray
ionization (ESI)(including, but not limited to,
15   nanoelectrospray ionization and/or capillary
electrospray), inductively coupled plasma (ICP), and
atmospheric pressure ionization (API)(including, but not
limited to, atmospheric pressure chemical ionization
(APCI)). Preferred ion sources include laser desorption,
20   thermospray (TSP), electrospray ionization (ESI),and
atmospheric pressure ionization (API). Suitable mass
analyzers include quadrupolar analyzers, quadrupole ion
trap or quistor analyzers, time-of-flight (TOF)
analyzers, magnetic and electromagnetic analyzers, and
25   ion cyclotron resonance analyzers, and Fourier transform
mass analyzers. The quadrupolar analyzers are preferred,
especially in connection with electrospray ionization
(e.g., a triple quadrupole analyzer). The time-of-flight
analyzers are more preferable in the context of the
30   present invention, with the most preferred devices for
producing and detecting fragments in the context of the
present invention being Matrix Assisted Laser Desorption
Ionization Time-of-Flight (MALDI-TOF) mass spectrometers.
Other mass spectrometers that can be used in the context
35   of the present invention include an electrospray
ionization mass spectrometer, a plasma desorption
spectrometer, a thermospray ionization spectrometer, and

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a laser desorption mass spectrometer. A spectrometer
that combines these techniques also can be suitable under
appropriate conditions. For example, recent advances in
the field have allowed the production of spectrometers
5   which combine electrospray ionization with time-of-flight
(or MALDI-TOF) analysis. One skilled in the art will
recognize that the present invention can readily be
practiced with such a spectrometer.

One skilled in the art will further recognize that
10   improved resolution and faster analysis can sometimes be
obtained using tandem mass spectrometry. Additionally,
by using tandem mass spectrometry (e.g., by using a
spectrometer possessing two detectors and a reflectron),
identification of monoisotopic masses versus average
15   masses is possible.

The fragments can have any suitable size for
detection and sample signal production and analysis.
Relatively large fragments can be detected upon initial
fragmentation, without further fragmentation. For
20   example, the produced and detected fragments can have
molecular weights of up to 5,000 Daltons (i.e., atomic
mass units or amu), 30,000 Daltons, 150,000 Daltons, or
even 300,000 Daltons or more. Thus, multiple
fragmentation events can be avoided prior to fragment
25   detection or sample signal production and analysis.

Similarly, the fragments can have any suitable mass-
to-charge ratio for detection and sample signal
production and analysis. Fragments with relatively large
mass-to-charge ratios can be detected, and sample signals
30   produced and analyzed therefor. For example, the
produced and detected fragments can have mass-to-charge
ratios (m/z) of at least 100, 200, 400, 500, 1,000,
2,500, 5,000, 10,000, 20,000, 25,000, or even 300,000 or
more.
35   The sample signal generated upon detection of the
fragments can be any type of sample signal that allows
for comparison to a standard signal. The sample signal

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can be generated in any suitable manner. Similarly, the
standard signal can be any signal and in any form that
allows for useful comparison with the sample signal to
evaluate the relative purity of the viral gene transfer

5   vector stock. The standard signal can be a single
standard signal or a series or group of standard signals
indicative of a range of viral gene transfer vector stock
purity levels. The sample and standard signals can be
associated with any suitable single mass-to-charge ratio

10   and/or any suitable range(s) of mass-to-charge ratios.
The sample signal and standard signal can be presented in
similar or different (though preferably similar) formats,
measurements, or units as long as a useful comparison can
be performed. For example, a suitable standard signal

15   can be a signal that is produced from techniques similar
to those that are used to generate the sample signal.
More specifically, the standard signal can be a signal
that is generated from a standard source, e.g., a viral
gene transfer vector stock of a known and/or desired

20   relative purity.
        The purity of the viral gene transfer vector stock
is evaluated by comparing the sample signal to the
standard signal in any suitable manner. For example,
sample signal and standard signal traces of mass-to-

25   charge ratio versus counts can be compared with respect
to the presence, absence, and/or nature of one, some, or
all of the peaks of the traces, which differences in the
traces reflects the relative purity of the viral gene
transfer vector stock.

30   The present method also can detect and characterize
(e.g., identify) one or more contaminants in the viral
gene transfer vector stock. For example, the present
method can detect and characterize contaminants in the
viral gene transfer stock by comparison of the standard

35   and sample signals. The contaminant can have any
suitable molecular weight, such as a molecular weight of
at least 1,000 Daltons, 3,000 Daltons, 5,000 Daltons,

10,000 Daltons, 30,000 Daltons, 100,000 Daltons, 200,000
Daltons, 500,000 Daltons, or even 1,000,000 Daltons or
more. Furthermore, the contaminant can have any suitable
mass-to-charge ratio (m/z), such as a mass-to-charge

5   ratio of at least 100, 200, 400, 500, 1,000, 2,500,
5,000, 10,000, 20,000, or even 25,000 or more.
       The contaminants can be any suitable type of
undesired molecule or composition that can be detected,
including, but not limited to, components of the viral

10 gene transfer vector stock. For example, the contaminant
can be any organic or inorganic molecule, compound, or
polymer, or a portion thereof. More specifically, the
contaminant can be a protein, nucleic acid,
polysaccharide, or a lipid.

15   Upon detection of one or more contaminants, the
contaminant(s) can be characterized by any suitable
technique. For example, the contaminant can be
characterized by comparison with the standard signal, or
by other techniques such as, but not limited to,

20 subjecting the contaminant to chromatography,
differential gradient analysis (e.g., a cesium chloride
gradient column or sucrose gradient), mass spectrometry,
electrophoresis, centrifugation, or any combination of
the aforementioned or other techniques.

25   Moreover, the contaminant(s) can be removed from the
aliquot or viral gene transfer vector stock. This can be
accomplished by any suitable technique, such as by any of
the techniques referenced above.
       In one aspect of the invention, the characterization

30 of the contaminant (or contaminants) identified can be
achieved through subjecting one or more fragments
corresponding to the contaminant to further fragmentation
and analysis. In this aspect, charged fragments are
produced from a fragment of the contaminant (i.e.,

35 secondary charged contaminant fragments). The secondary
charged contaminant fragment then can be detected by a
detector that produces a signal corresponding to the

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mass-to-charge ratio of the detected secondary
contaminant fragment. This signal (e.g., a spectrum)
corresponding to the secondary charged contaminant
fragment allows rapid identification of the contaminant.

5   Any suitable technique or combination of techniques
can be used to produce charged fragments from the
contaminant fragment, detect the charged secondary
contaminant fragment, and generate a signal corresponding
to the secondary charged contaminant fragment. For

10   example, the contaminant fragment can be contacted with
protons, electrons, or radiation to produce the secondary
charged contaminant fragment or, preferably, be subjected
to collision induced disassociation (CID). Detection of
the secondary charged contaminant fragment is preferably

15   performed by the use of an analytical device, most
preferably by the use of an ion source and a mass
analyzer, such as are present in mass spectrometers.
More preferably, a MALDI-TOF spectrometer with precursor
selector (or "ion gate") capabilities, a quadrupole

20   electrospray spectrometer, or any spectrometer capable of
focussing on one or more signals that correspond to the
contaminant (or contaminants) in the stock is used to
produce, detect, and analyze the secondary charged
contaminant fragment. A MALDI-TOF spectrometer with

25   precursor selector capabilities is particularly preferred
because it is capable of accurately detecting (and
focusing) on a signal corresponding to the contaminant,
isolating the contaminant fragment, and rapidly
performing further analysis on the isolated contaminant

30   fragment.
        An example of this aspect of the invention is the
identification of the amino acid composition of a
polypeptide contaminant by the above technique. After
identifying a signal (or signals) corresponding to the

35   polypeptide contaminant, the polypeptide can be further
fragmented (e.g., by contact with radiation). By
controlling fragmentation, fragments corresponding to

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individual constituent amino acids can be produced.
Signals corresponding to the amino acids then can be
generated, allowing rapid determination of the amino acid
composition of the polypeptide.

5   A similar technique can be used as a method to
provide more detailed information regarding the purity of
the viral gene transfer vector within the stock. For
example, one or more signals within the sample signal
that correspond to a fragment of the viral gene transfer

10   vector (i.e., a standard fragment) can be identified.
The standard fragment then can be further fragmented
(e.g., by subjecting the standard fragment to radiation)
to produce at least one secondary charged standard
fragment from the standard fragment. The secondary

15   charged standard fragment can be detected by a detector
which produces a signal corresponding to the mass-to-
charge ratio of the detected secondary standard
fragments. This signal can allow identification of the
components of the standard fragment (e.g., the amino acid

20   composition of a viral protein of interest), similar to
the technique described above for identifying specific
contaminants. This technique can be particularly useful
in ensuring the presence (or absence) of certain gene
products in the viral gene transfer vector stock. While

25   any suitable combination of techniques can be used to
produce charged fragments from the standard fragment,
detect the secondary charged standard fragment, and
generate a signal corresponding to the secondary charged
standard fragment, the preferred techniques described

30   above relating to the contaminant fragment also are
applicable to this aspect of the invention.

         In some instances it can be desirable to combine
fragmentation (of the contaminant fragment or standard
fragment) with supplemental fragmentation techniques.

35   Suitable supplemental fragmentation techniques include
collision-induced disassociation (CID), post source decay
(PSD), and combinations thereof.

20

Accuracy can sometimes be improved by isolating the
contaminant fragment or standard fragment, or a signal
corresponding thereto, prior to further fragmentation.
For example, the present inventive method can include a
5   step of substantially isolating the contaminant fragment
before producing a secondary charged contaminant fragment
from the contaminant fragment. Alternatively a signal or
signals known to correspond to the contaminant fragment
can be isolated (i.e., from other signals in the sample
10   signal) and focused on before producing a secondary
charged contaminant fragment from the contaminant
fragment. Similar techniques can be applied to the
standard fragment. Any suitable method can be used to
isolate the contaminant fragment or standard fragment
15   prior to producing the secondary charged fragment.
Similarly, any suitable method can be used to isolate a
signal corresponding to the contaminant fragment or
standard fragment. Preferably, isolating a signal
corresponding to the contaminant fragment or standard
20   fragment is done by using a MALDI-TOF spectrometer with
ion trap capabilities, a quadrapole electrospray
spectrometer, or any spectrometer capable of focussing on
one or more signals corresponding to the contaminant
fragment or standard fragment.
25   Any of the individual steps of the present method
can be repeated two or more times. For example, the
production of fragments and the detection of the
fragments can be repeated two or more times to provide a
satisfactory sample signal(s). Similarly, multiple viral
30   gene transfer stocks can be provided, an aliquot can be
obtained from each such viral gene transfer vector stock,
and a sample signal can be produced for each such
aliquot, thereby enabling the selection of stocks that
have a desired purity based on comparison of the sample
35   signals to the standard signal.
The aliquots can be taken from stocks that have been
subjected to sequential cycles of separating the viral

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gene transfer vector from the production cell and medium.
After analysis of the sample signals by comparison with a
standard signal, as described above, stocks that have a
desired purity can be selected based on the comparison of
5   the sample signals to the standard signal. In this
fashion, for example, the viral gene transfer vector
stock purification process can be evaluated or
"benchmarked."

10   EXAMPLE
         This example further illustrates the present
invention but should not be construed to limit the
present invention in any way. Although this example is
recited using particular aspects of the invention, for
15   example, particular viral gene transfer vector stocks, as
well as fragmentation and detection techniques, the
skilled artisan will appreciate that the present
inventive method can be applied to a wide range of viral
gene transfer vector stocks, using a wide variety of
20   techniques as described above.
         Specifically, four different adenoviral vector
stocks were provided. The adenoviral vector in each
stock was an adenovirus of serotype 5 (i.e., Ad5) and
contained an exogenous gene. The carrier in each stock
25   was the same and was a physiologically compatible aqueous
solution. The adenoviral vector stocks differed as to
the manner in which each was purified from the production
cells and growth media. The first two adenoviral vector
stocks, Samples A and B, were prepared using standard
30   chromatography techniques. The third adenoviral vector
stock, Sample C, was prepared using three successive
cesium chloride gradients and was considered to represent
an adenoviral vector stock of a desirably high degree of
purity. The fourth adenoviral vector stock, Sample D,
35   was prepared using a nonconventional anion exchange
chromatography matrix technique.

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An aliquot was obtained from each of the four
adenoviral vector stocks. Each aliquot was treated to
reduce its salt concentration, thereby reducing the noise
in the subsequent sample signal. Each aliquot was
5   subjected to MALDI-TOF mass spectrometry so as to produce
and detect fragments from the aliquot. The mass
spectrometry settings were such that the produced
fragments comprised substantially intact proteins
disassociated from the adenoviral vector, rather than
10   portions of those proteins. The sample signal produced
from the detection of the produced fragments resulted in
a mass spectrometer trace for each of the aliquots of the
four adenoviral vector stocks. These mass spectrometer
traces are set forth in Figure 1 in a graph of mass-to-
15   charge ratio (m/z) (x-axis) versus relative counts (y-
axis). Since the third adenoviral vector stock, Sample
C, reflected the desired level of purity of the
adenoviral vector, the Sample C mass spectrometer trace
represented the "standard signal" in accordance with the
20   present invention for purposes of considering the purity
of the other adenoviral vector stocks, Samples A, B, and
D. The Sample A, B, and D mass spectrometer traces were
treated as the "sample signals" in accordance with the
present invention.
25   As is apparent from the mass spectrometer traces of
Figure 1, the fourth adenoviral vector stock, Sample D,
is approximately as pure as the "control" adenoviral
vector stock, Sample C. The other adenoviral vector
stocks, Samples A and B, are not as pure as the "control"
30   adenoviral vector stock, Sample C, with Sample B being
less pure than Sample A. This is readily apparent from a
comparison of the traces, e.g., the presence, absence,
and nature of the peaks of the traces.
In the Sample C "standard signal," the peaks at, for
35   example, approximately 11000, 12000, and 14500 mass-to-
charge ratios (m/z) are quite sharp. This is also true
with respect to the Sample D "sample signal" as compared

to the Sample C "standard signal." Moreover, there are
no significant additional or missing peaks in the Sample
D "sample signal" as compared to the Sample C "standard
signal." This similarity of the Sample D "sample signal"
5 and the Sample C "standard signal" reflects a similar
degree of purity of the adenoviral vector stocks.

    In contrast, the peaks at approximately 11000,
12000, and 14500 mass-to-charge ratios (m/z) are much
broader in the Sample A and Sample B "sample signals" as
10 compared to the Sample C "standard signal." Moreover,
there are significant additional peaks in the Sample A
"sample signal" at, for example, approximately 17500 and
23000 mass-to-charge ratios (m/z), and in the Sample B
"sample signal" at, for example, approximately 11500 and
15 13500 mass-to-charge ratios (m/z), as compared to the
Sample C "standard signal." In addition, there are
significant missing peaks in the Sample A and Sample B
"sample signals" at, for example, 4500 mass-to-charge
ratio (m/z), as compared to the Sample C "standard
20 signal." This lack of similarity of the Sample A "sample
signal" and the Sample C "standard signal," and the even
greater lack of similarity of the Sample B "sample
signal" and the Sample C "standard signal," reflect the
presence of a higher level of contaminants in those
25 adenoviral vectors stocks (i.e., adenoviral vector stocks
of lower purity), more so with respect to Sample B than
Sample A, as compared to the "control" adenoviral vector
stock Sample C.

    Thus, it is readily apparent from the results of
30 this example that the present invention is quite useful
in assessing the relative purity of viral gene transfer
vector stocks.

    All of the references cited herein, including
35 patents, patent applications, and publications, are hereby
incorporated in their entireties by reference.

   While this invention has been described with an
emphasis upon preferred embodiments, it will be obvious to
those of ordinary skill in the art that other variations
of the invention can be used and that it is intended that
5   the invention can be practiced otherwise than as
specifically described herein. Accordingly, this
invention includes all modifications encompassed within
the spirit and scope of the invention as defined by the
following claims.

WHAT IS CLAIMED IS:

1.   A method of evaluating the purity of a viral gene transfer vector stock, the method comprising:

5   (a)   providing an aliquot of a viral gene transfer vector stock;

(b)   producing charged fragments from the aliquot;

(c)   detecting the fragments by a detector which produces a sample signal corresponding to the mass-to-

10   charge ratio of the detected fragments;

(d)   providing a standard signal; and

(e)   evaluating the purity of the viral gene transfer vector stock by comparing the sample signal to the standard signal.

15

2.   The method of claim 1, wherein providing an aliquot of a stock of a viral gene transfer vector in step (a) of the method comprises:

(a1) transfecting a viral gene transfer vector into

20   a cell that is permissive for the replication of the viral gene transfer vector to produce a transfected cell, wherein the cell is in a medium;

(a2) maintaining the transfected cell in the medium so as to cause the viral gene transfer vector to

25   replicate;

(a3) separating the viral gene transfer vector from the cell and from the medium to produce a stock of a viral gene transfer vector; and

(a4) obtaining an aliquot from the stock.

30

3.   The method of claim 2, wherein the method further comprises:

(a5) repeating steps (a3) and (a4) a desired number of times to obtain a series of viral gene transfer vector

35   stocks and aliquots thereof;

(f)   performing steps (a)-(e) on each aliquot
obtained in step (a5) to produce a set of sample signals;
and

(g)   selecting stocks that have a desired purity
based on the comparison of the sample signals to the
standard signal.

4   The method of any of claims 1-3, wherein steps
(b) and (c) are repeated two or more times.

5.   The method of claim 4, wherein steps (b) and
(c) are performed by tandem mass spectroscopy.

6.   The method of any of claims 1-4, wherein steps
(b) and (c) are performed by a mass spectrometer selected
from the group consisting of an electrospray ionization
mass spectrophotometer, a plasma desorption
spectrophotometer, a thermospray ionization
spectrophotometer, and a laser desorption mass
spectrophotometer.

7.   The method of any of claims 1-4, wherein steps
(b)-(d) are performed by Matrix Assisted Laser Desorption
Ionization Time-of-Flight (MALDI-TOF) spectrophotometry.

8.   The method of any of claims 1-4, wherein
steps (b) and (c) are performed by a spectrometer
capable of electrospray ionization and time-of-flight
analysis.

9.   The method of any of claims 1-8, wherein the
viral gene transfer vector is an adenoviral vector.

10.   The method of any of claims 1-9, wherein the
viral gene transfer vector comprises proteins associated
with each other, and the fragments of the viral gene

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transfer vector comprise substantially intact proteins
disassociated from the viral gene transfer vector.

        11. The method of any of claims 1-10, wherein the
5   fragments of the viral gene transfer vector consist
    essentially of substantially intact proteins
    disassociated from the viral gene transfer vector.

        12. The method of any of claims 1-11, wherein the
10 comparison of the sample signal to the standard signal in
    step (e) further comprises the step of detecting the
    presence of a contaminant in the viral gene transfer
    vector stock.

15   13. The method of claim 12, wherein the method
    further comprises:
        (h) characterizing the contaminant.

        14. The method of claim 13, wherein the
20 characterization of the contaminant in step (h) of the
    method comprises:
        (h1) identifying at least one signal in the sample
    signal corresponding to a contaminant fragment;
        (h2) producing at least one secondary charged
25 contaminant fragment from the contaminant fragment;
        (h3) detecting the secondary charged contaminant
    fragments by a detector which produces a signal
    corresponding to the mass-to-charge ratio of the
    detected secondary charged contaminant fragments.
30

        15. The method of any of claims 12-14, wherein the
    method further comprises the step of removing the
    contaminant from the viral gene transfer vector stock.

35   16. The method of any of claims 1-15, wherein the
    viral gene transfer vector has a molecular weight at
    least 100,000,000 Daltons.

17.   The method of any of claims 1-16, wherein the viral gene transfer vector is a vector that contains more than 4,000 nucleotides or nucleic acid base pairs.

18.   The method of any of claims 1-17, wherein the sample signal comprises a signal produced from a fragment having a molecular weight of up to 300,000 Daltons when the fragment is detected.

19.   The method of any of claims 1-18, wherein the sample signal comprises a signal produced from a fragment having a mass-to-charge ratio of at least 200 when the fragment is detected.

20.   The method of any of claims 1-19, wherein the standard signal is provided by a viral gene transfer vector stock of known purity.

21.   The method of any of claims 1-20, wherein the standard signal is provided in the same manner as the sample signal.

22.   The method of any of claims 1-21, wherein the fragments pass through a field-free region, wherein the velocity of the fragments within the field-free region is proportional to the mass-to-charge ratio of the fragments.

23.   The method of claim 22, wherein the detection of the fragments in step (c) further comprises:
        (c1)   measuring the time-of-flight for the detected fragments, wherein the time-of-flight is the approximate time required for a fragment to travel a distance across the field-free region; and
   (c2) producing a sample signal corresponding to the time-of-flight.

24. The method of any of claims 1-23 wherein step
(e) of the method further comprises:

   (e1) identifying at least one signal in the sample
5 signal corresponding to a standard fragment;

   (e2) producing at least one secondary charged
standard fragment from the standard fragment; and

   (e3) detecting the secondary charged standard
fragments by a detector which produces a signal
10 corresponding to the mass-to-charge ratio of the
detected secondary charged standard fragments.

FIG. 1
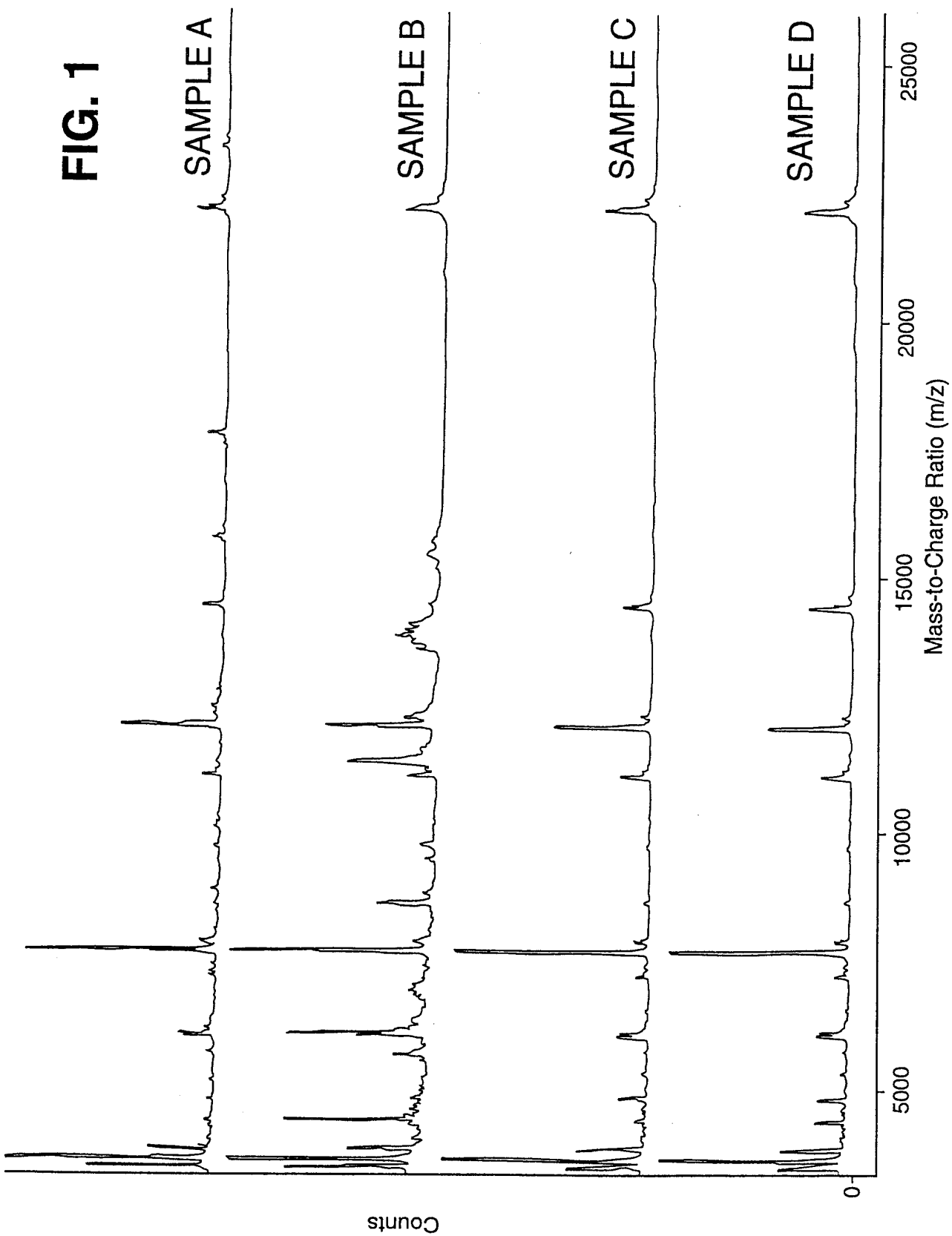
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7   C12Q1/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7   C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SIUZDAK: "PROBING VIRUSES WITH MASS SPECTROMETRY" J.MASS.SPECTROM., vol. 33, March 1998 (1998-03), pages 203-211, XP000863342 cited in the application the whole document & DATABASE HCA 'Online! RAPID COMMUN. MASS SPECTROM., 1995 FUERSTENAU ET AL.: "MOLECULAR WEIGHT DETERMINATION OF MEGADALTON DNA ELECTROSPRAY IONS USING CHARGE DETECTION TIME-OF-FLIGHT MASS SPECTROMETRY" abstract ——— -/--	1-24

| X | Further documents are listed in the continuation of box C. | | X | Patent family members are listed in annex. |

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
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Name and mailing address of the ISA	Authorized officer
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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